

# Yeast Surface Display as an Emerging Technology for Biofuel Production

*“Transforming yeasts into whole-cell biocatalysts: looking at its role in establishing the bio-based economy”*

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”

Looking at one of the BIGGEST problems that the world is facing today...

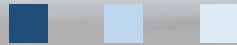


# Climate Change

*“Climate change is moving faster than we are ... We must listen to the Earth’s best scientists.”*

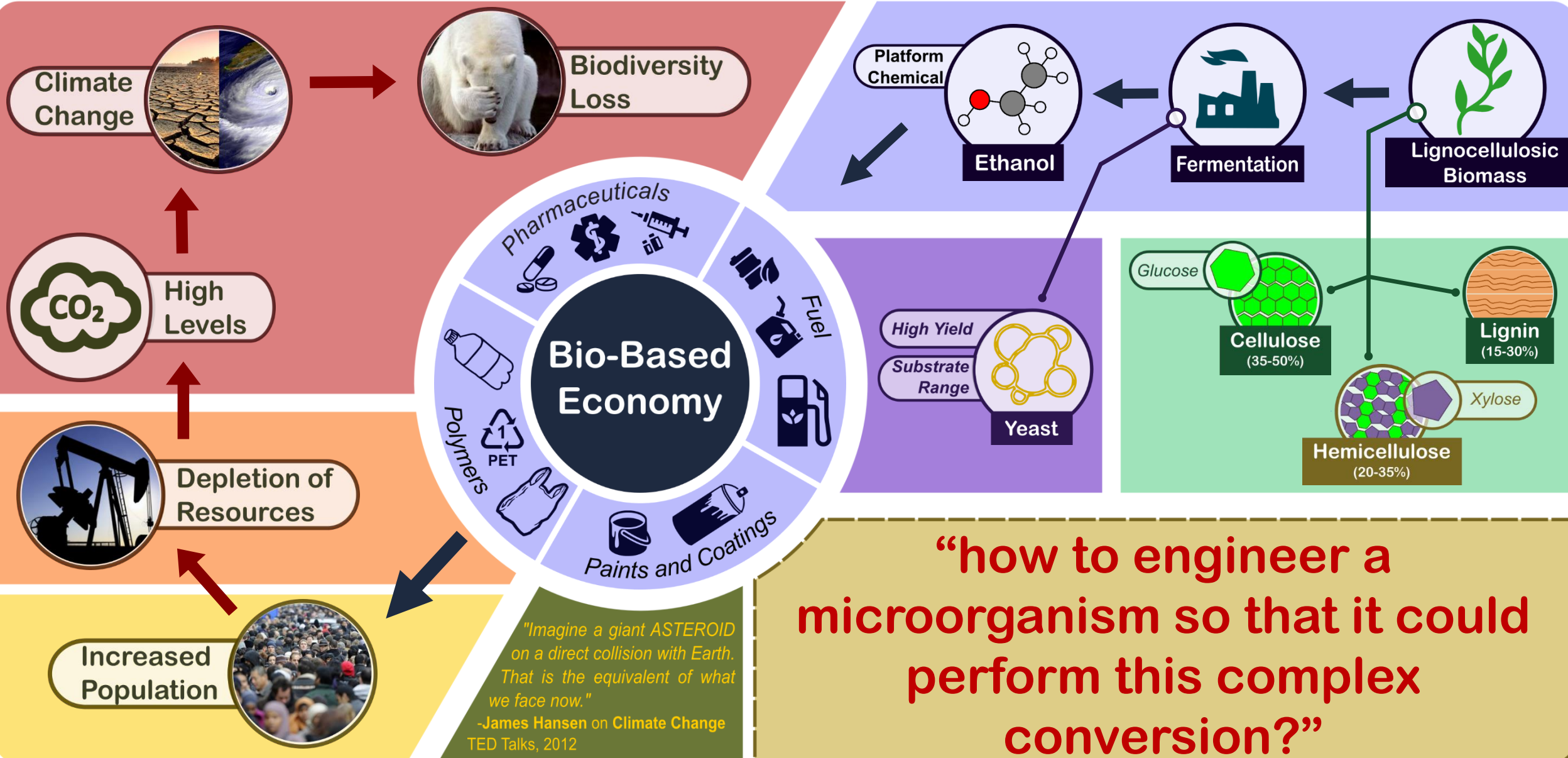
**-António Guterres**  
UN Secretary General

*Adapted from his 2018 UN General Assembly address*





# ENGINEERING YEAST: A QUEST AGAINST CLIMATE CHANGE



# The Biorefinery Concept

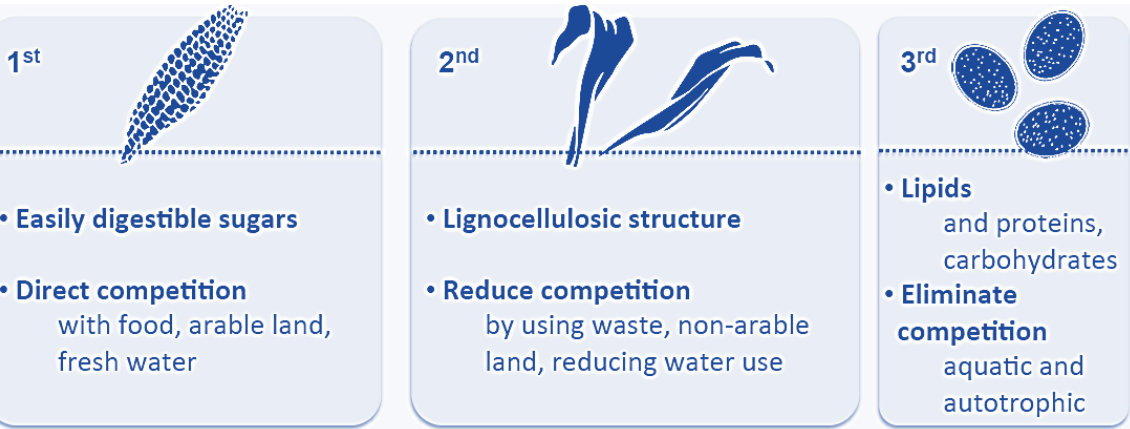
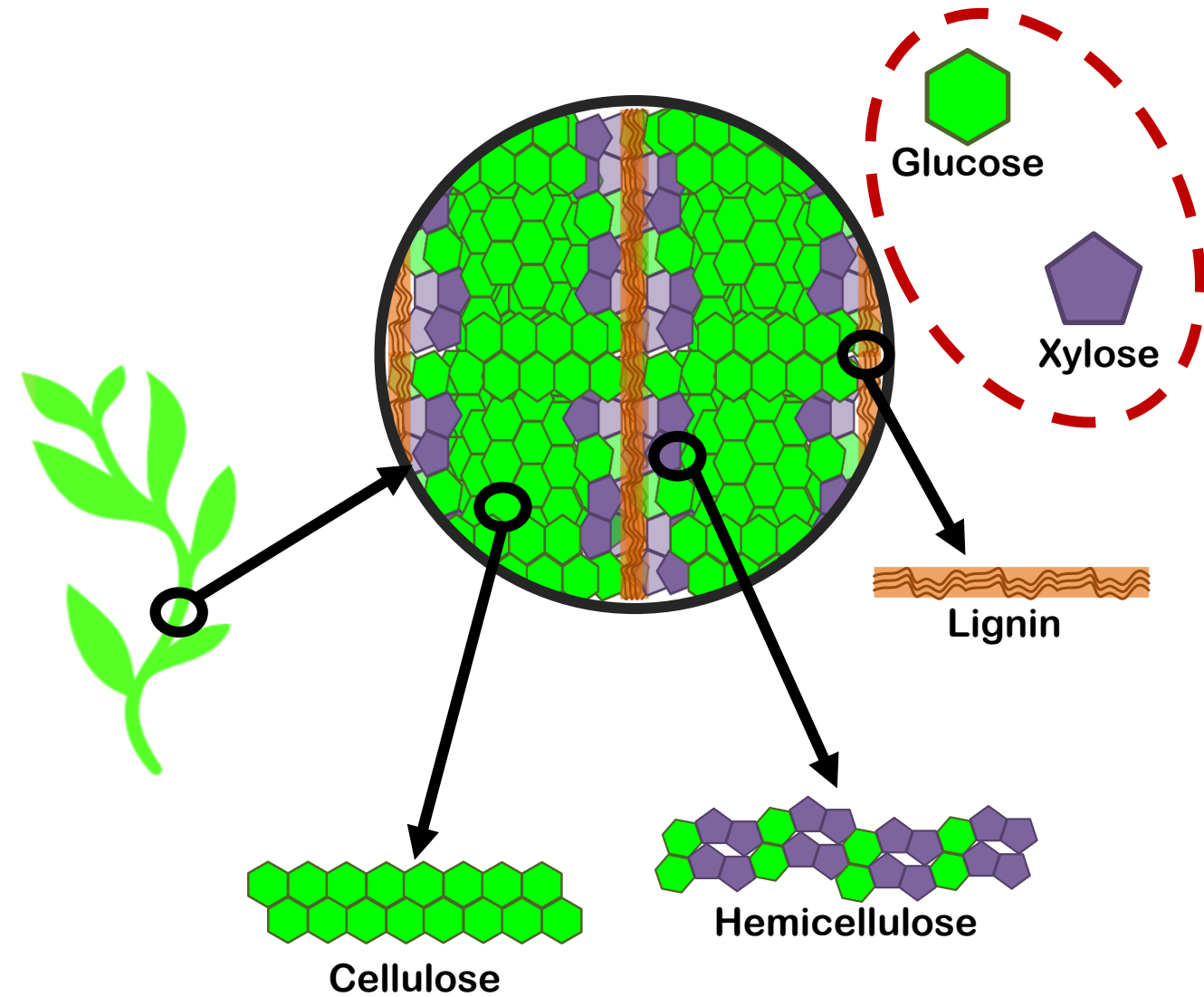
*“A sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat).”*



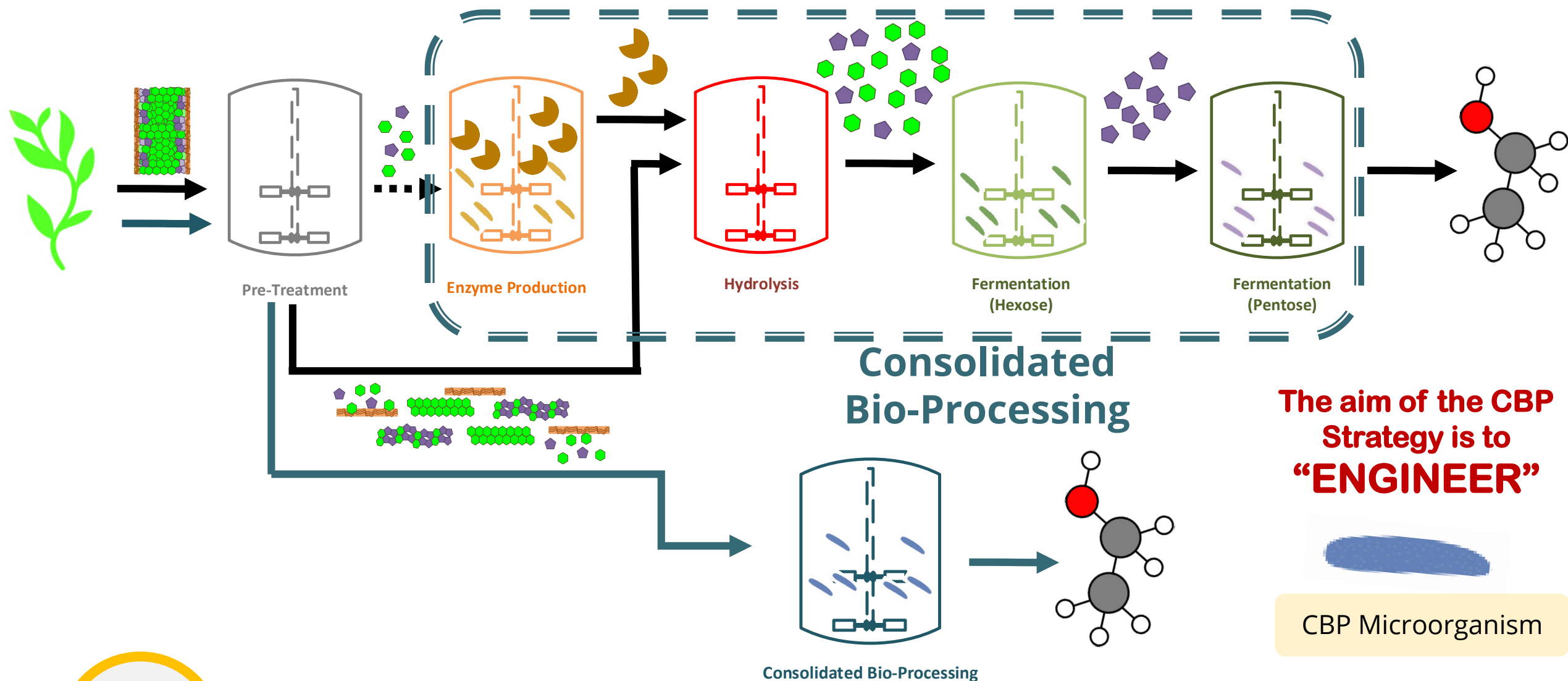
**-IEA Bioenergy Task 42**  
On “Biorefinery Definition”







**Biomass Feedstocks:** the raw material of the bio-based economy



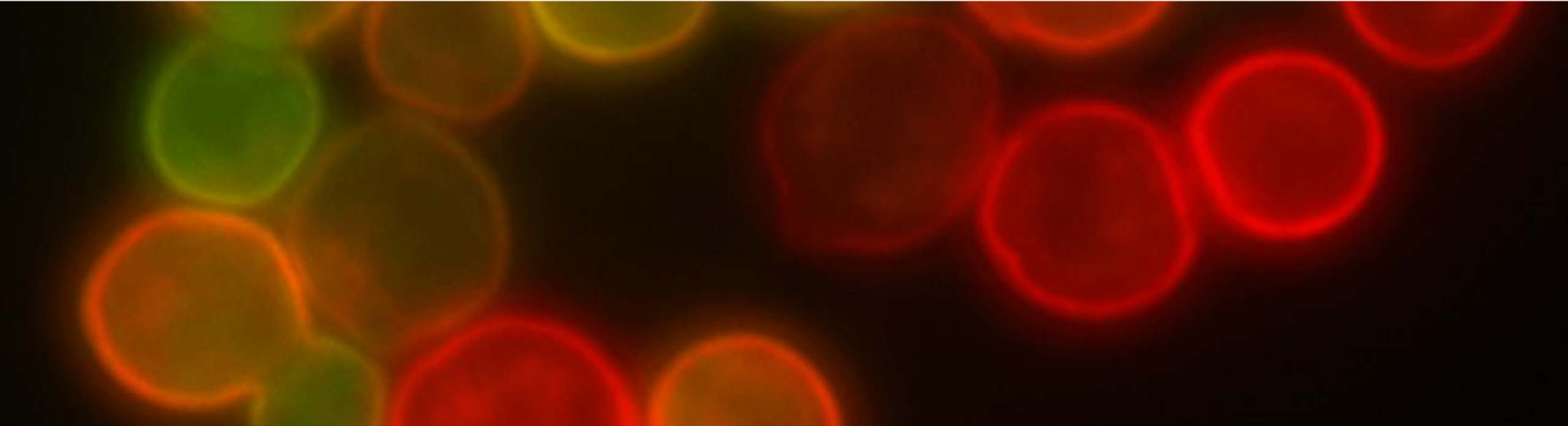
**Bioprocessing:** converting biomass into platform chemicals for further production of bio-based products

From lignocellulosic biomass to bioethanol ...

# Yeast Cell Surface Display in Lignocellulosic Bioethanol Production

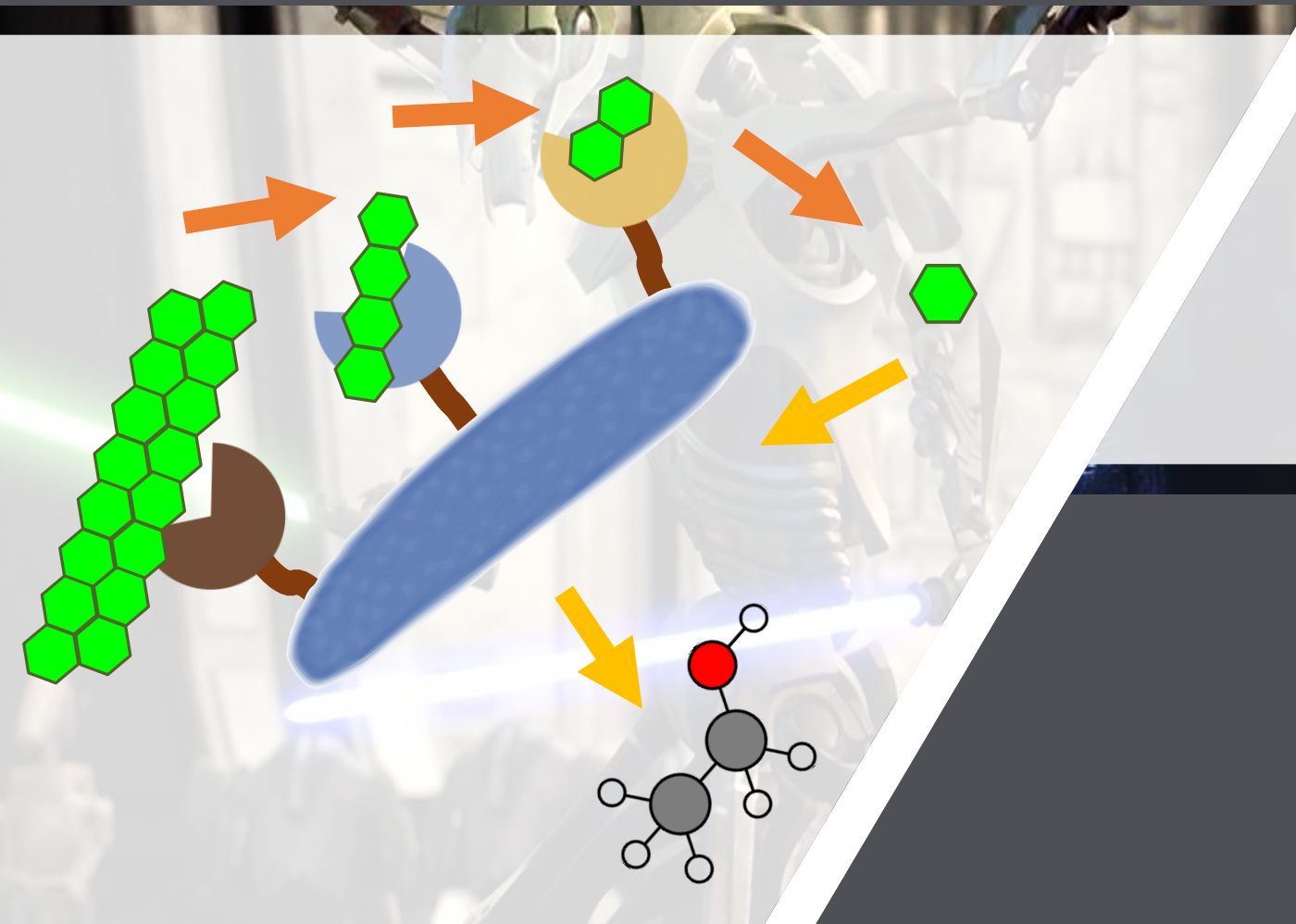
*“this field has elevated the status of *S. cerevisiae* as a novel and attractive microorganism to be a platform for enzyme immobilization which enables it to target nonconventional substrates and at the same time, renewable self-immobilized biocatalysts (a.k.a. whole-cell biocatalysts) .”*

-Ueda and Tanaka, 2000  
J. Biosci. Bioeng

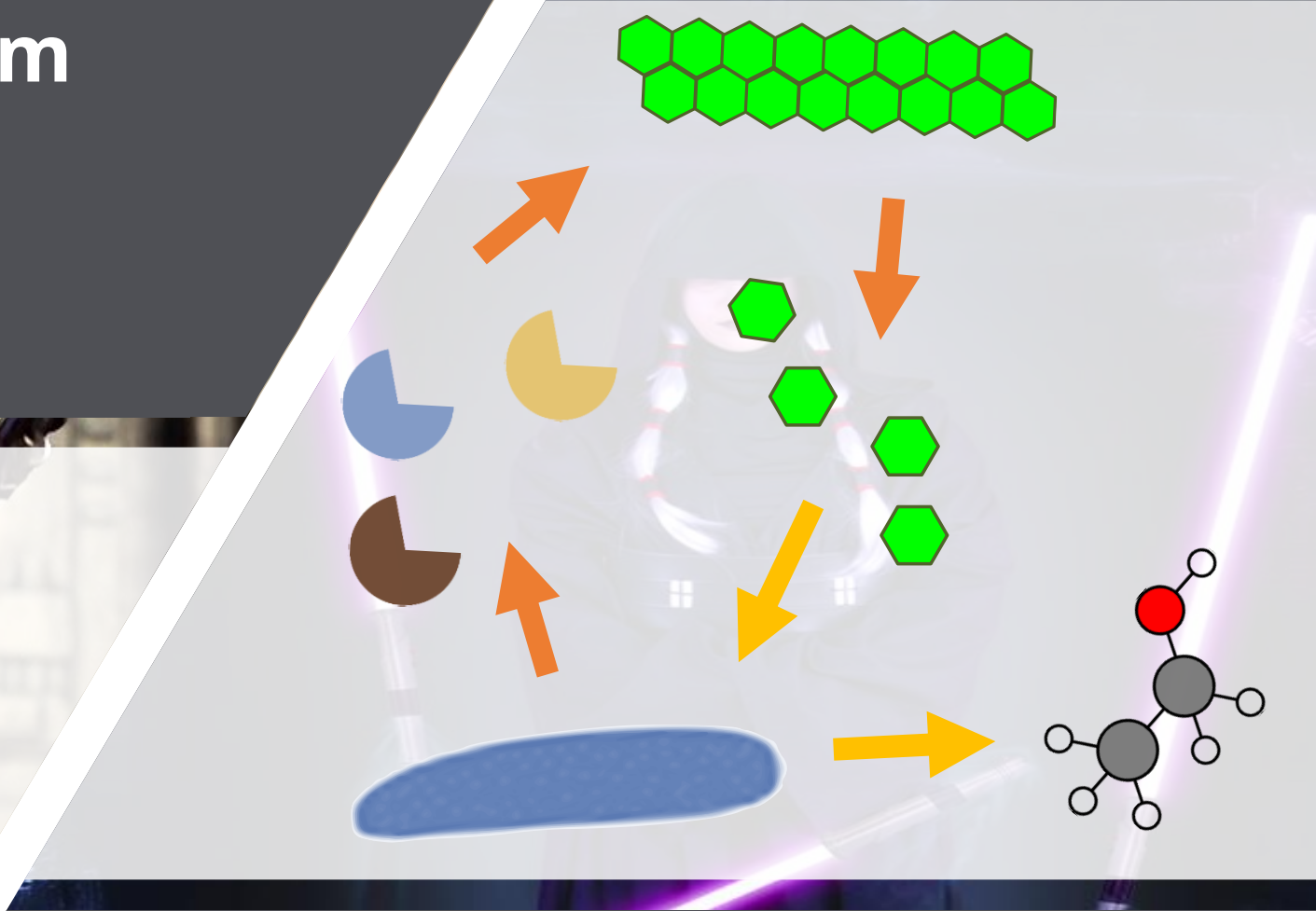
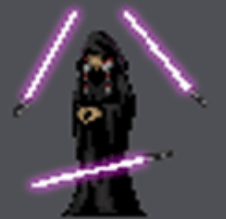




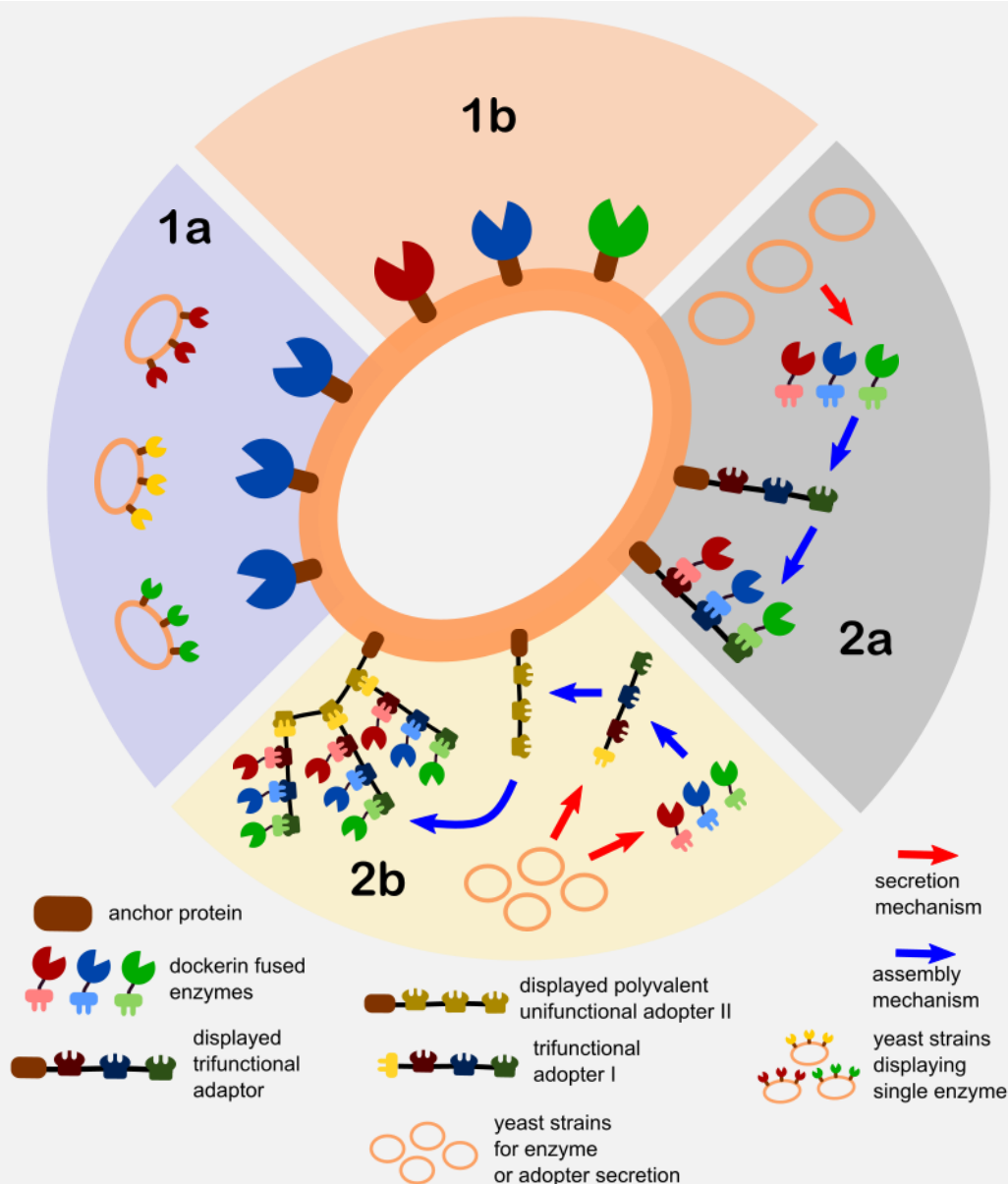
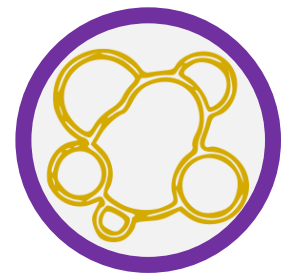
# Immobilized Enzyme System



# Secretion Enzyme System



# How is this platform exploited for Bioethanol Production?



## Direct Yeast Surface Display (DYSD)

*directly involves the fusion of the enzyme of interest/s to the yeast anchor protein for it to be expressed and localize on the cell surface*

**1a – Single Enzyme Display Consortium (SEDC)**

**1b – Co-Display (CD)**

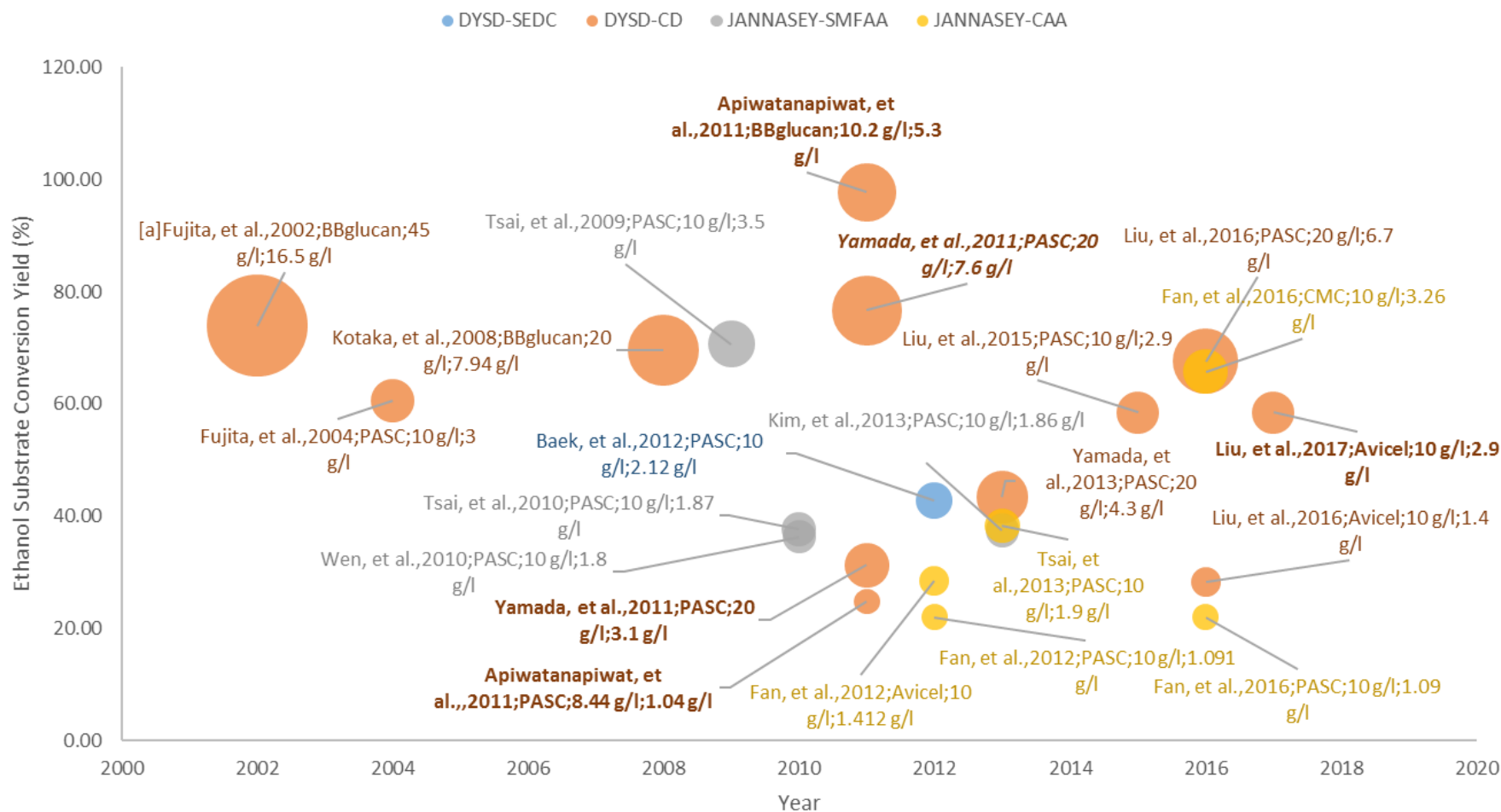
## Juxtaposed Assembly of Non-Native Adaptors and Secreted Enzymes on Yeast (JANNASEY)

*developed based on the biomimicry of the “cellulosome” machinery of several anaerobic cellulose-consuming microorganisms (e.g., clostridia, ruminal bacteria)*

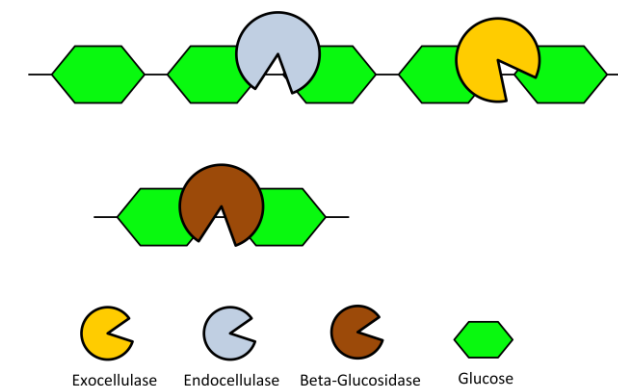
**2a – Single Multi-Functional Adaptor Assembly (SMFAA)**

**2b – Complex Adaptor Assembly (CAA)**



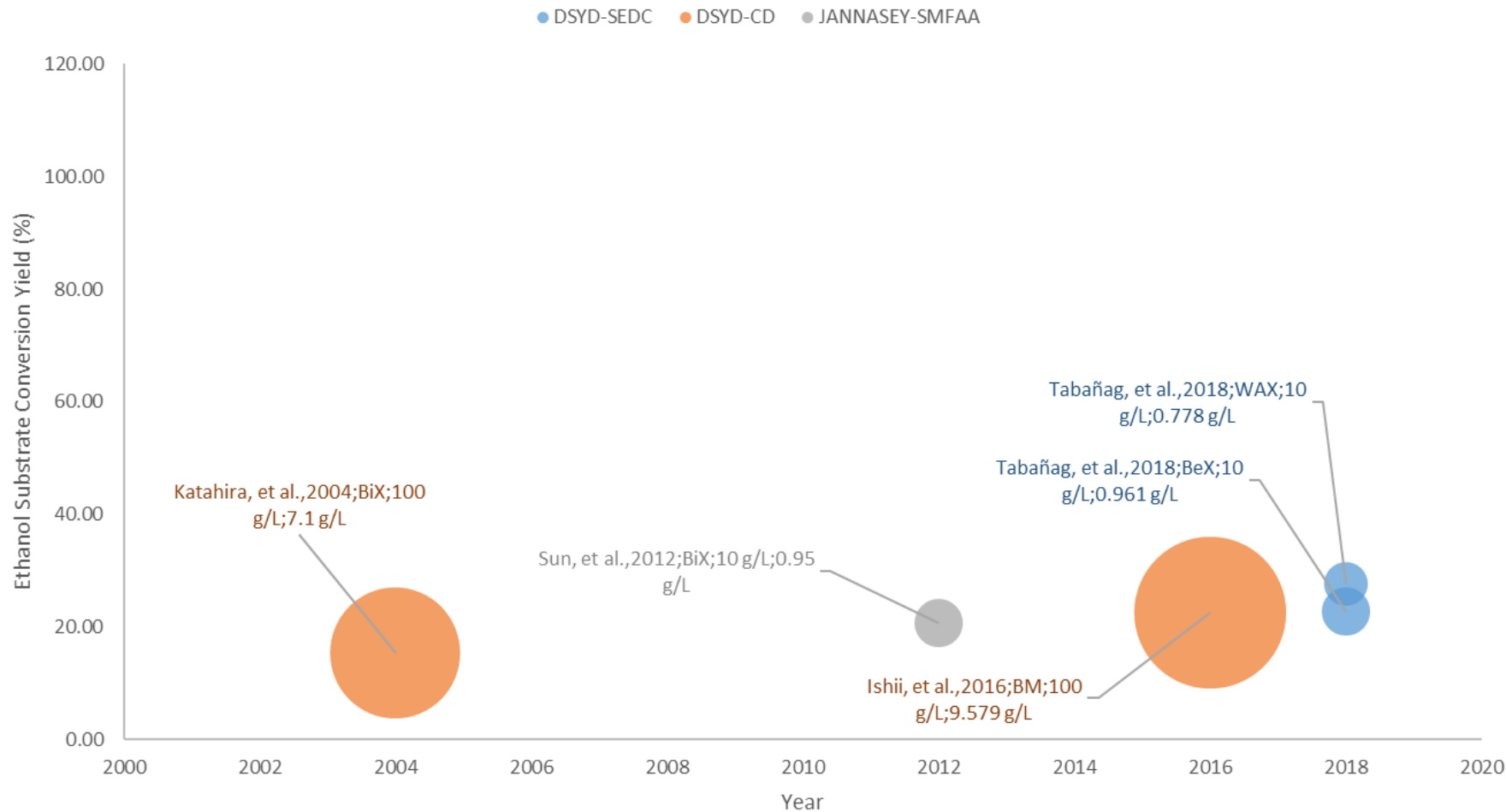


Visualization of the status and development of yeast surface display for Cellulosic Bioethanol Production. Bubble sizes represent ethanol titer. Data labels are formatted as follows: (Reference (author-date); Substrate; Substrate Concentration; Ethanol Titer). For the DYSD-CD data set: labels in boldface represent cellulase ratio control via delta-integration; labels in italic represent utilization of diploid host strains.

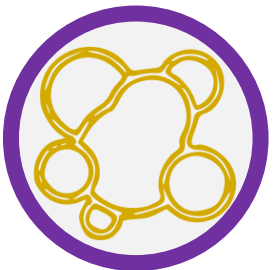
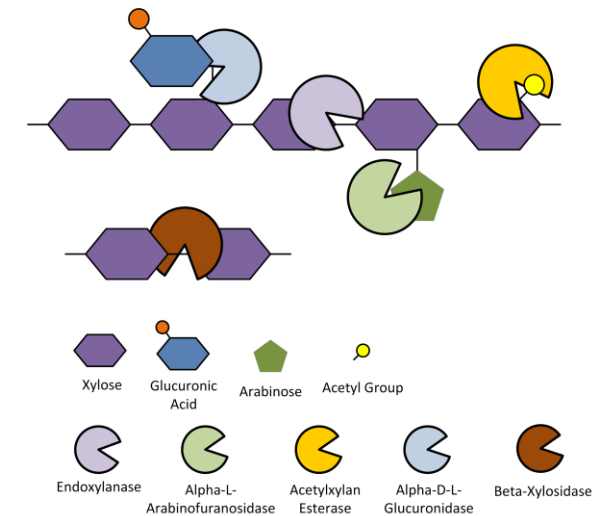


Tabañag, I. D. F., I.-M. Chu, Y.-H. Wei and S.-L. Tsai (2018). "The Role of Yeast-Surface-Display Techniques in Creating Biocatalysts for Consolidated BioProcessing." *Catalysts* 8(3): 94.

## YSD and Bioethanol Production from Pure Cellulose Substrates



Visualization of the status and development of yeast surface display for Hemicellulosic Bioethanol Production. Bubble sizes represent ethanol titer. Data labels are formatted as follows: (Reference (author-date); Substrate; Substrate Concentration; Ethanol Titer).

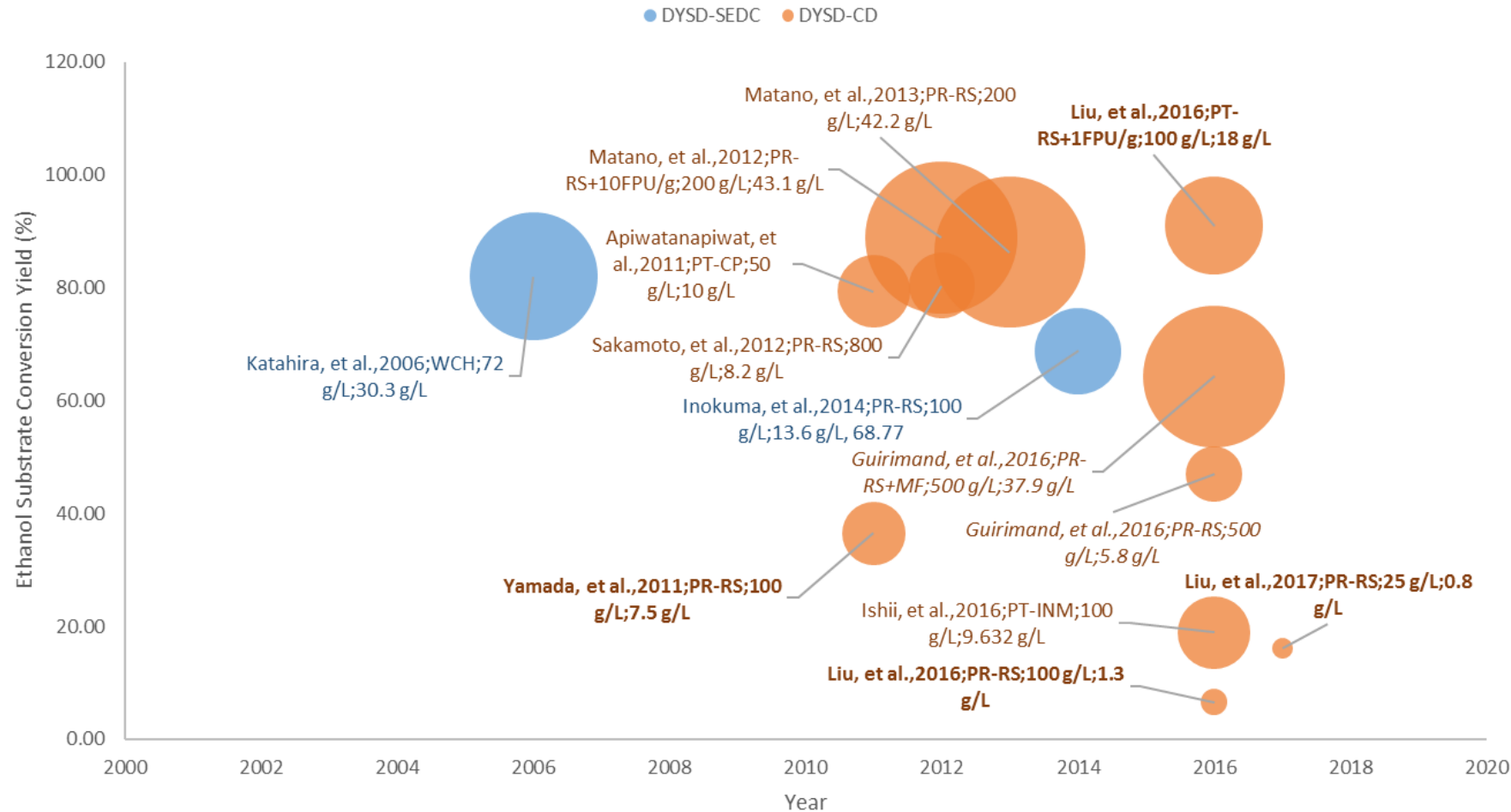
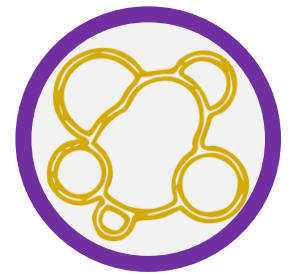


Tabañag, I. D. F., I.-M. Chu, Y.-H. Wei and S.-L. Tsai (2018). "The Role of Yeast-Surface-Display Techniques in Creating Biocatalysts for Consolidated BioProcessing." *Catalysts* 8(3): 94.

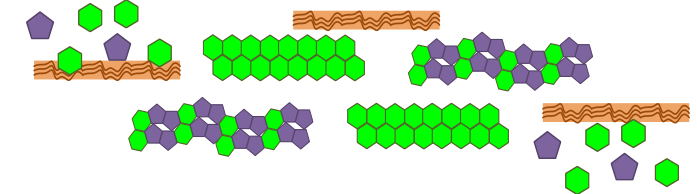
## YSD and Bioethanol Production from Pure Hemicellulose (Xylan) Substrates



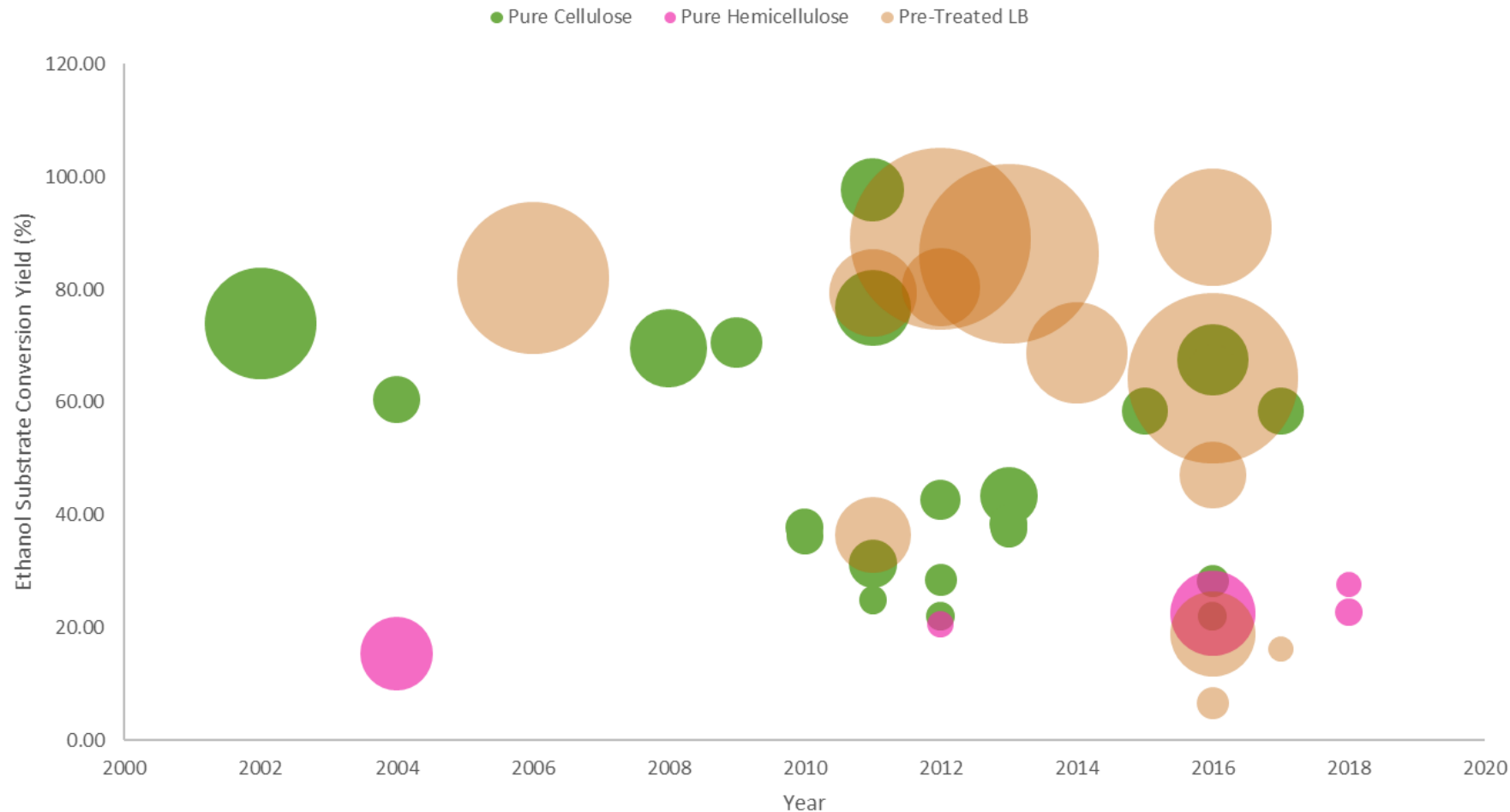
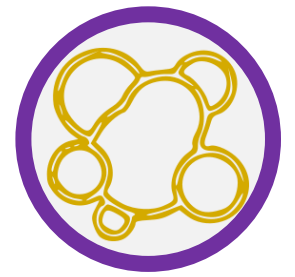
# YSD and Bioethanol Production from Pre-Treated LB Substrates



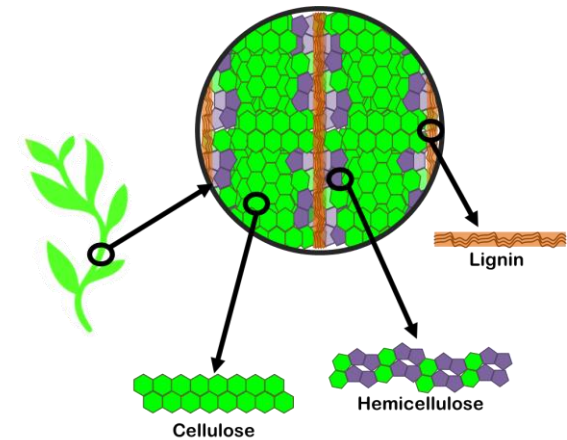
Visualization of the status and development of yeast surface display for Bioethanol Production from Pre-Treated Lignocellulosic Biomass. Bubble sizes represent ethanol titer. Data labels are formatted as follows: (Reference (author-date); Substrate; Substrate Concentration; Ethanol Titer). For DYSD-CD data sets: labels in boldface represent cellulase ratio control via delta-integration; labels in *italic* represent the xylitol fermentation parameters instead of ethanol.



# A perspective on the extent of YSD application to various substrates



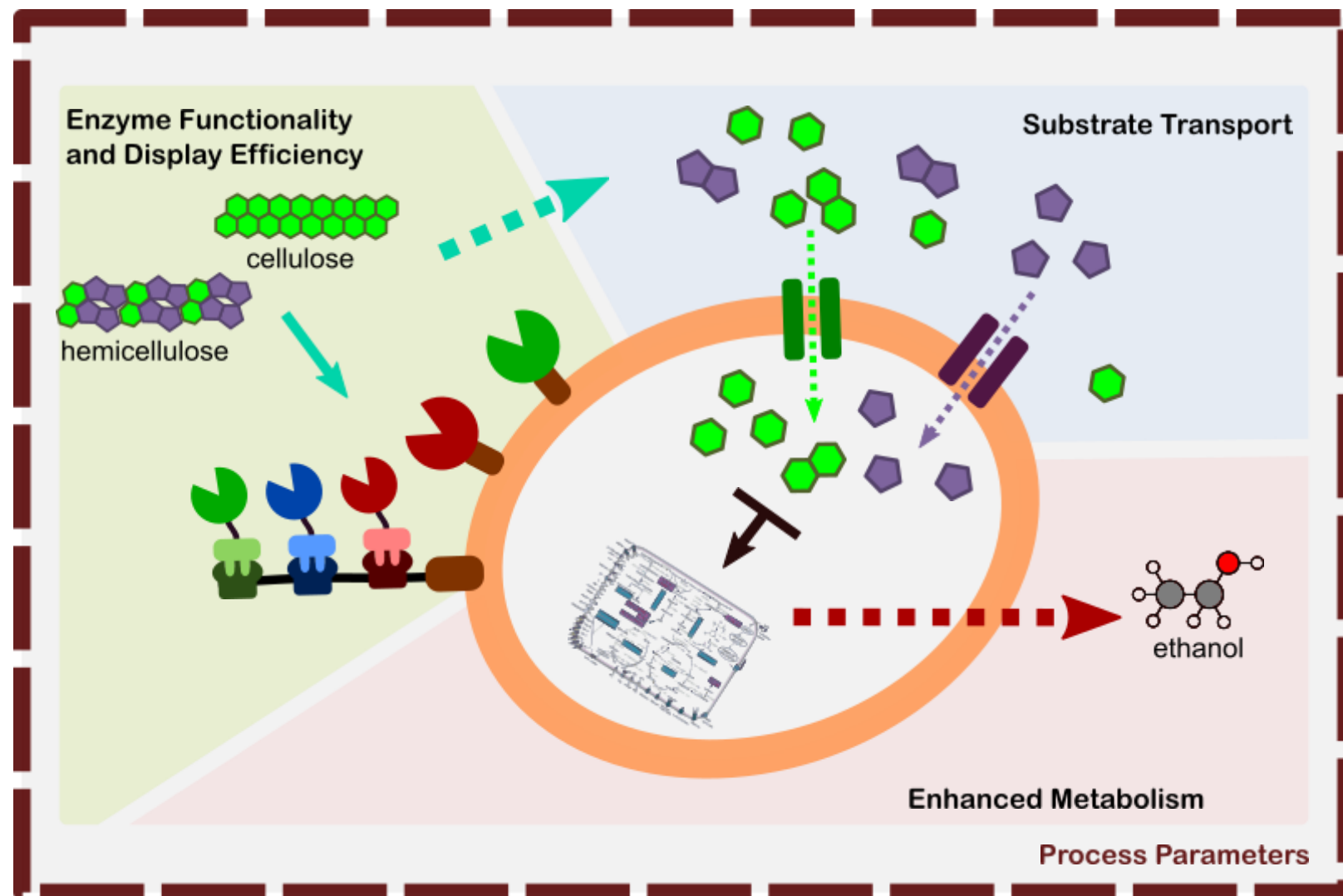
Visualization of the status and development of yeast surface display for Bioethanol Production from Pre-Treated Lignocellulosic Biomass. Bubble sizes represent ethanol titer. Data labels are formatted as follows: (Reference (author-date); Substrate; Substrate Concentration; Ethanol Titer). For DSYD-CD data sets: labels in boldface represent cellulase ratio control via delta-integration; labels in *italic* represent the xylitol fermentation parameters instead of ethanol.





# "The Role of Yeast Surface Display in Bioethanol Production from Lignocellulosic Substrates"

*On the Variables that Affect the Strain Performance*



Tabañag, I. D. F., I.-M. Chu, Y.-H. Wei and S.-L. Tsai (2018). "The Role of Yeast-Surface-Display Techniques in Creating Biocatalysts for Consolidated BioProcessing." *Catalysts* 8(3): 94.

On the avenues of improvement



# Contextualizing

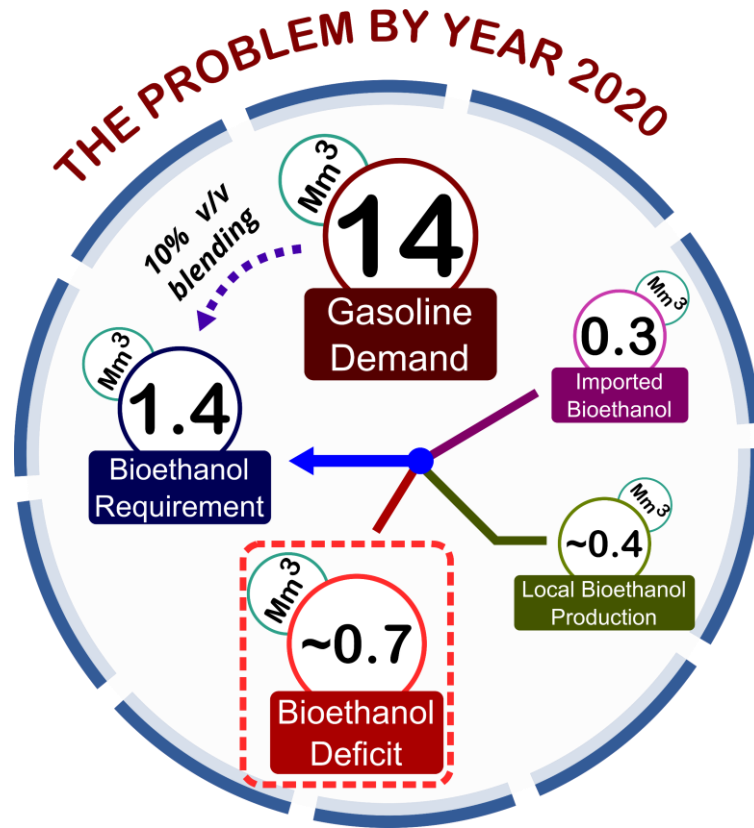
*“Looking at the application of the consolidated bio-processing strategy on the production of biofuels in the Philippines using Agro-Industrial Residues”*



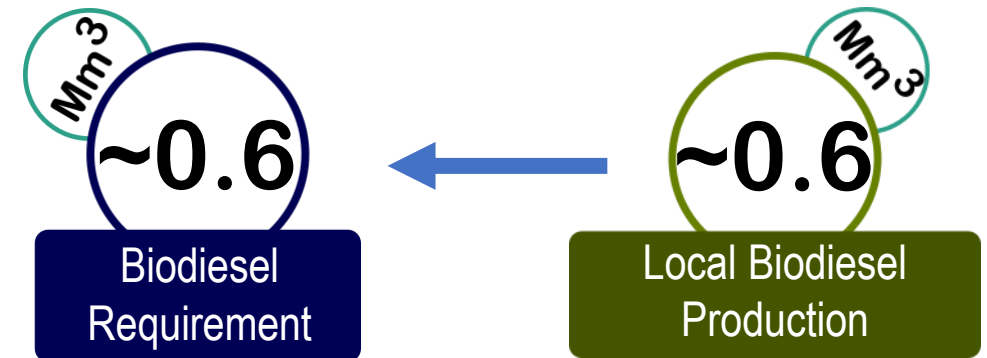


# The Problem

## Bioethanol Scenario (*based on 2020 projected demand*) [1]



## Biodiesel Scenario (*based on 2020 demand*) [2]

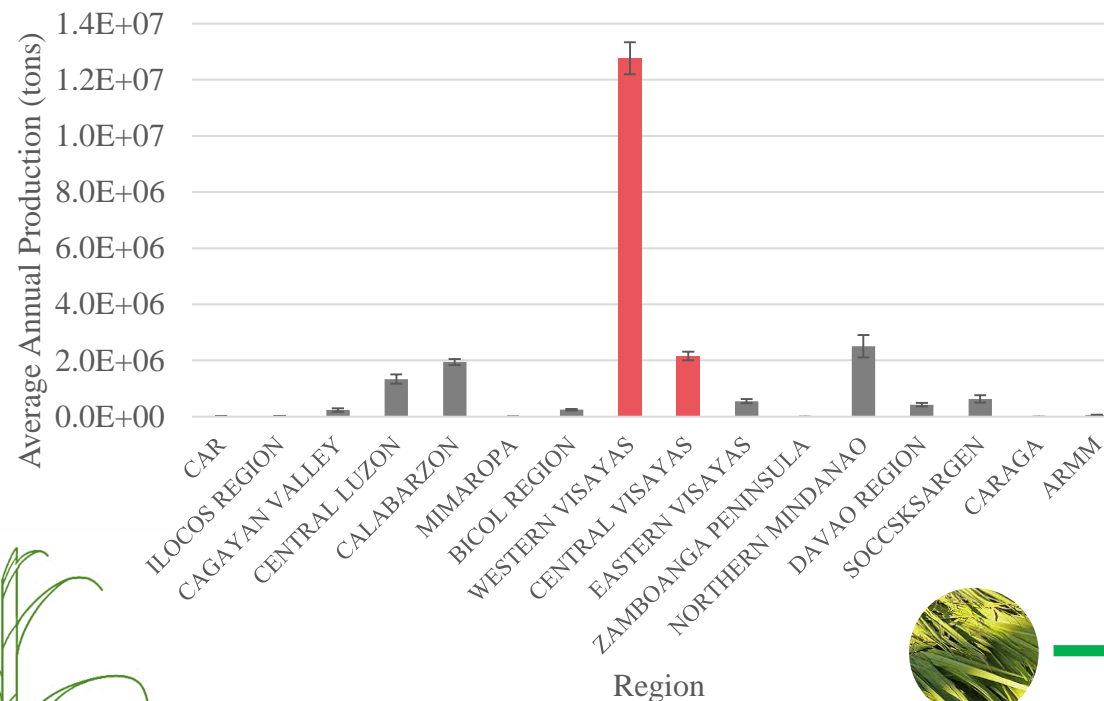
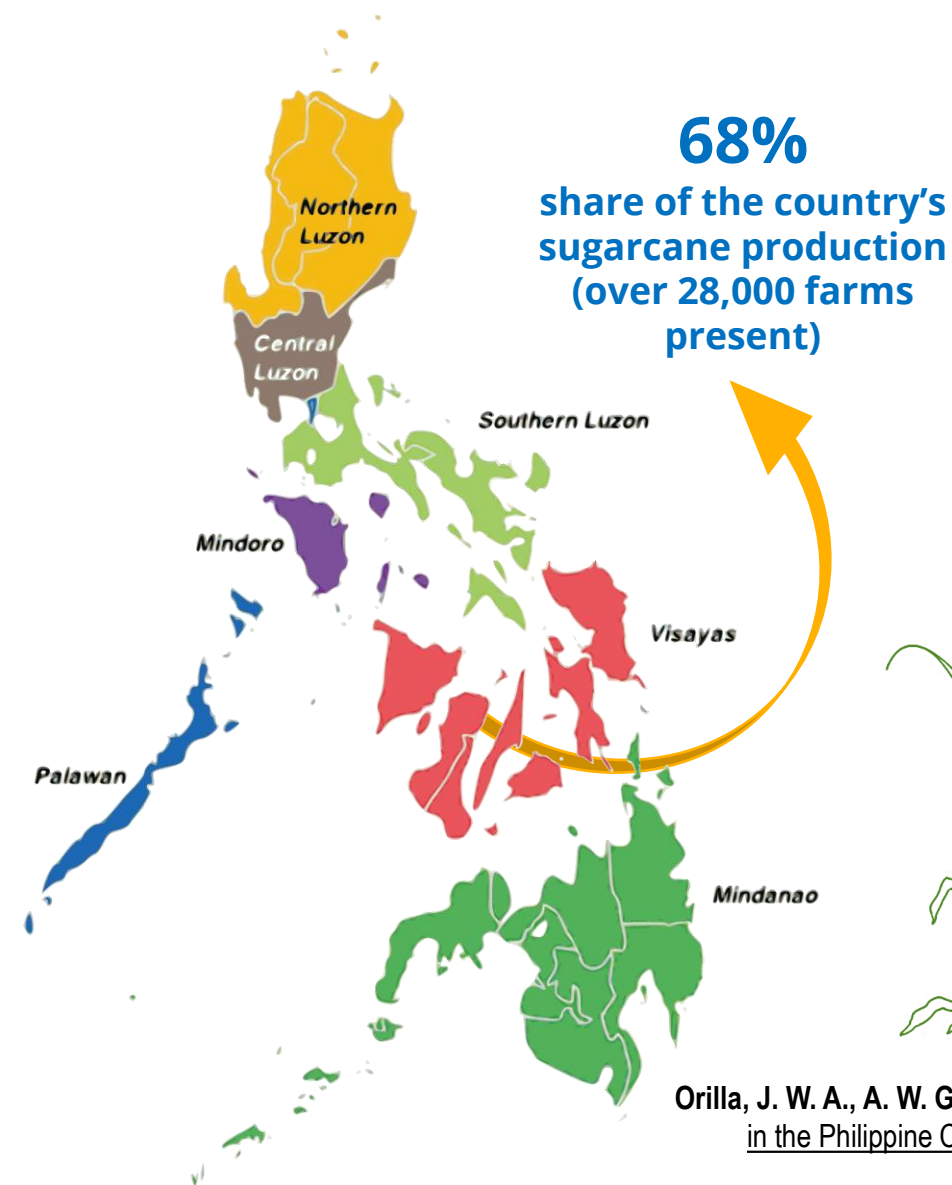


# FOOD vs FUEL conundrum

[1] - Orilla, J. W. A., A. W. Go, A. T. Conag, A. S. Toledo, Y. H. Ju and I. D. F. Tabañag (2019). *Valorization of Sugarcane Residues for the Production of Bioethanol in the Philippine Context: A Retrospective Assessment from 2007-2017 and Future Challenges*. 2019 National Research Council of the Philippines (NRCP) Annual Scientific Conference, Manila, Philippines. *Manuscript Submitted for Publication*

[2] – Adapted from the presentation of Dr. R.B. Demafilis

# Looking at what we have...



**Average annual sugarcane  
production in the  
Philippines:  $22.7 \pm 1.1$   
Million Tons/year**



**$3.43 \pm 1.1$  Mt of  
dry SCL/yr**



**$3.17 \pm 1.1$  Mt of  
dry SCB/yr**



**$0.92 \pm 0.05$  Mt of  
molasses/yr**

Orilla, J. W. A., A. W. Go, A. T. Conag, A. S. Toledo, Y. H. Ju and I. D. F. Tabañag (2019). Valorization of Sugarcane Residues for the Production of Bioethanol in the Philippine Context: A Retrospective Assessment from 2007-2017 and Future Challenges. 2019 National Research Council of the Philippines (NRCP)

Annual Scientific Conference, Manila, Philippines. Manuscript Submitted for Publication

# On addressing our country's need for biofuels

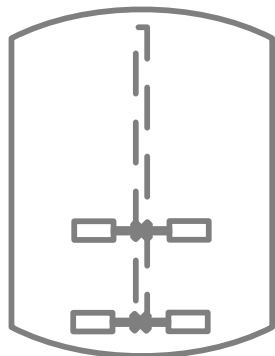
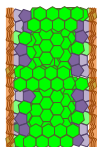
3.43 ± 1.1 Mt of dry  
SCL/yr

1.44 ± 0.6 Mt/yr

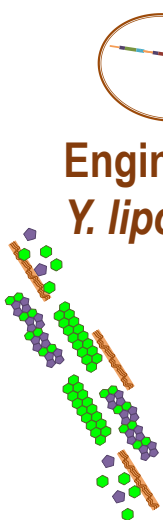
glucose

1.09 ± 0.5 Mt/yr

xylose



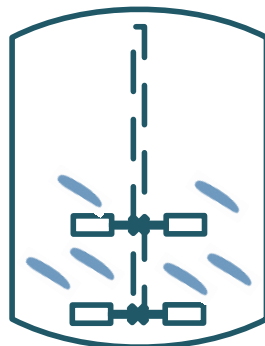
Pre-Treatment



Engineered  
*Y. lipolytica*

~0.27  $\frac{g \text{ biodiesel}}{g \text{ glucose}}$

~0.17  $\frac{g \text{ biodiesel}}{g \text{ xylose}}$



Consolidated Bio-Processing



Downstream Processing



Biodiesel



Bioethanol

~0.58 ± 0.3 Mm<sup>3</sup>/yr  
Biodiesel from SCL

~0.58 ± 0.3 Mm<sup>3</sup>/yr  
Biodiesel SCB

3.17 ± 1.1 Mt of dry  
SCB/yr

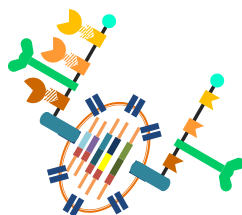
1.44 ± 0.7 Mt/yr

glucose

1.02 ± 0.5 Mt/yr

xylose

~0.51  $\frac{g \text{ ethanol}}{g \text{ glucose or xylose}}$



Engineered  
*S. Cerevisiae*

~1.47 ± 0.7 Mm<sup>3</sup>/yr

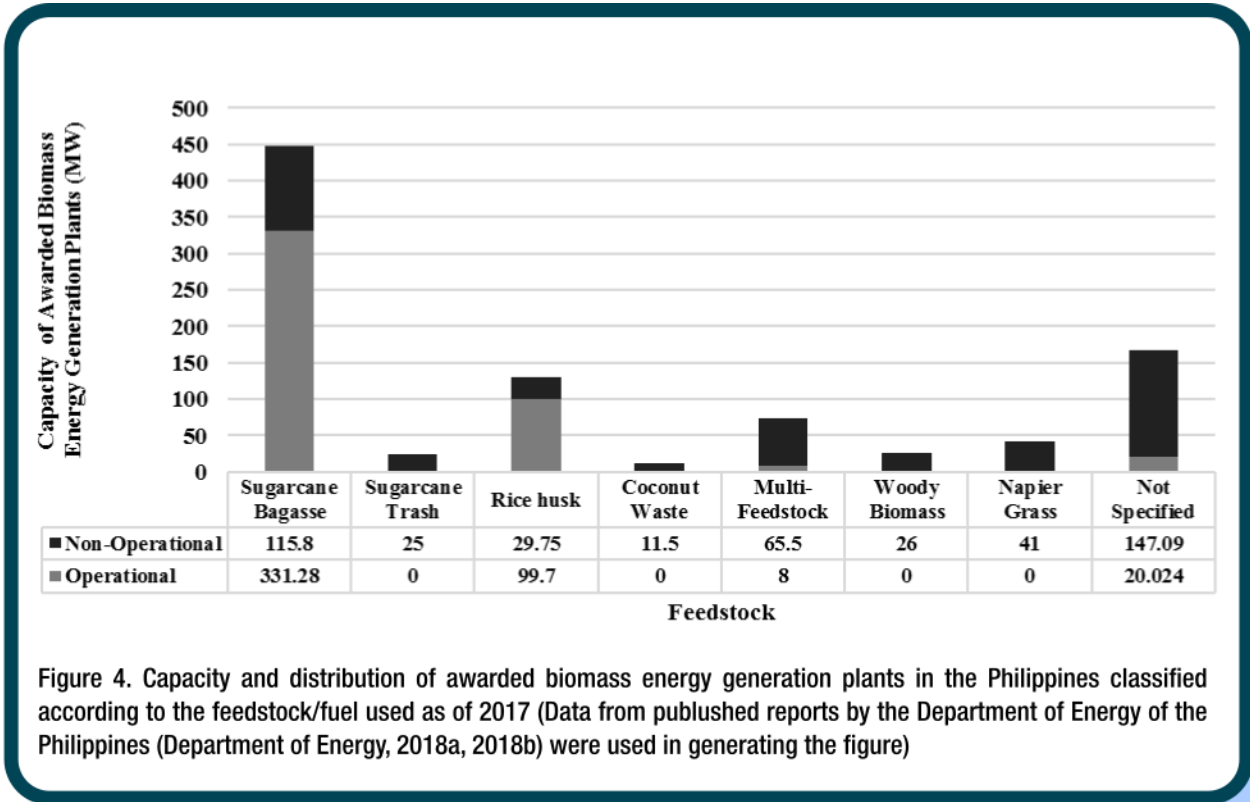
Bioethanol from

SCL

~1.43 ± 0.7 Mm<sup>3</sup>/yr

Bioethanol SCB





3.43 ± 1.1 Mt  
of dry  
SCL/year



3.17 ± 1.1 Mt  
of dry  
SCB/year



0.92 ± 0.05 Mt of  
molasses/year

Percentage of the available SCB  
used as solid fuel rather than  
feedstock for biofuel production

77%

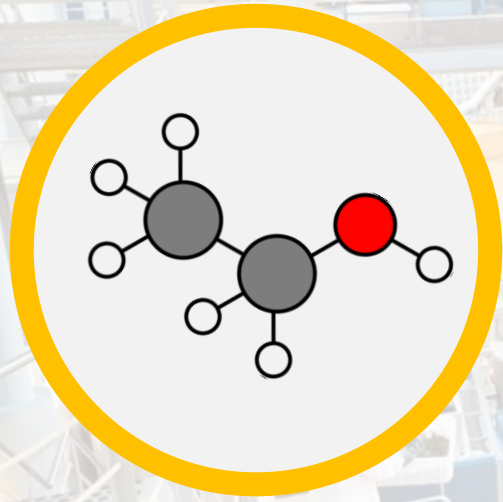
# The Challenge:

*On spreading the awareness that this "underutilized feedstock" can be "valorized" to produce "high-value products" that can set the foundations of a bio-based economy*

What are we doing with these sugarcane residues?



# Bio-Based Platform Chemicals

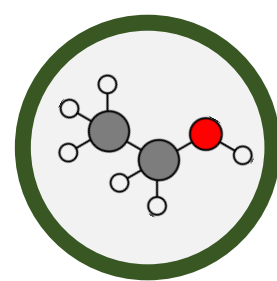


A prelude to “**Bio-based Products**”





# Taking a peek at the bio-based platform chemicals production



Industrial chemicals and materials produced using only biological reactions. This simplified map covers 92 out of 435 chemicals and materials (boxed) presented in the poster (downloadable through the QR code). Solid and dotted arrows indicate single and multiple reaction steps, respectively. The abbreviated enzyme names indicate enzymes with a single substrate specificity (blue) and those with a broad substrate range (red). Glucose is used as a representative carbon source towards the production of industrially relevant chemicals and materials. Xylose is another carbon source that is also often considered for the production of ethylene glycerol and poly(lactate-co-glycolate). The fatty acid biosynthetic pathway is presented as a circle to indicate a series of fatty acid chain elongation reactions.



Bio-Based  
Chemicals Map



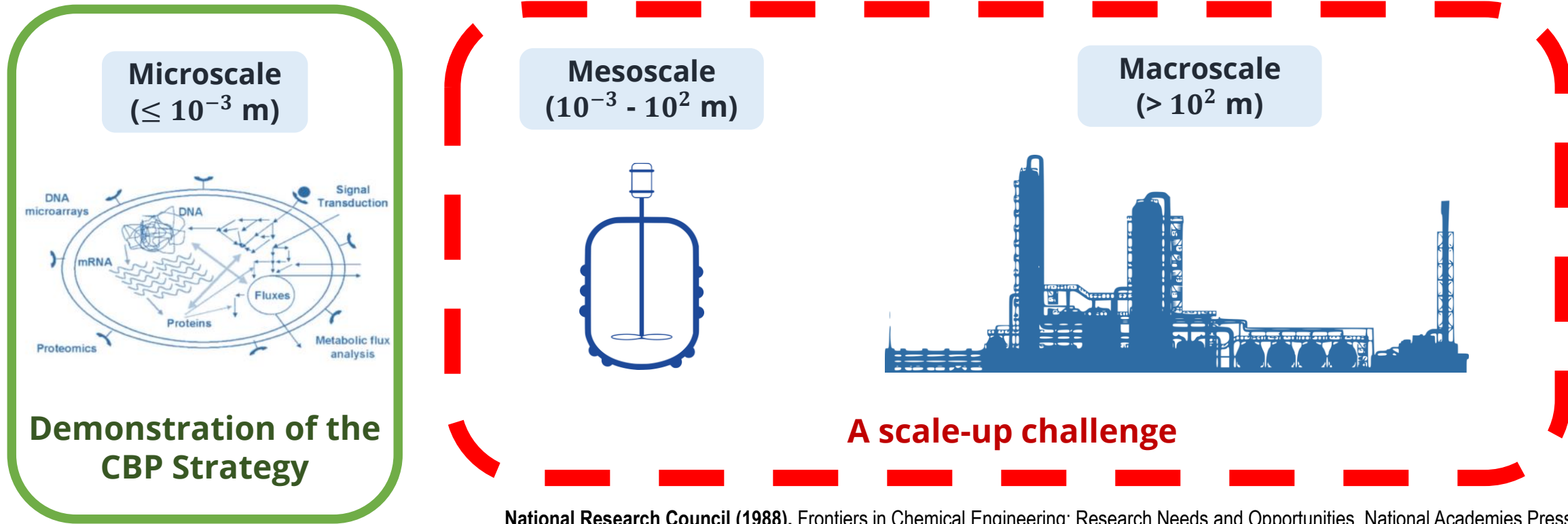
Bio-Based  
Chemicals Paper



“

Chemical engineers conceive and rigorously solve problems on a ‘**continuum**’  
of **scales**’ ranging from **microscale** to **macroscale**

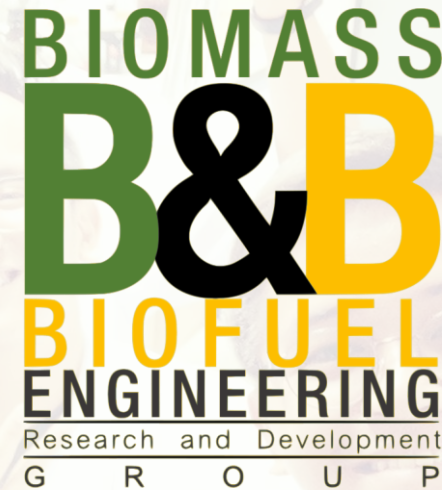
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Looking at the CBPstrategy through the perspective of a Chemical Engineer

**As a research group we see that we are...**

- A community of researchers bridging knowledge gaps and developing technologies on biomass utilization and biofuel production.
- A relevant research group influencing national policies and laws facilitating the development of a profitable industry based on biomass utilization and biofuel production.



**As a research group, we...**



- Promote education and research towards an effective and efficient use of local biomass resources for a sustainable bio-based industry.
- Provide scientifically sound, innovative alternatives and solutions in biomass processing for the benefit of the local, national, and global communities.



**Thank you for  
your attention!**

*Any comments  
or questions?*